

**Use of Microarray Data in Support
of a *Hypothetical* Drug Submission:
Attenuation of Ventricular Remodeling
Associated with Heart Failure
in an Animal Model**

Thomas Papoian, Ph.D., D.A.B.T.

Pharmacologist / Toxicologist
Division of Cardiovascular and Renal Products
Center for Drug Evaluation and Research
U.S. Food and Drug Administration

October 6, 2005
DIA/FDA/PhRMA/BIO/PWG Workshop
Bethesda, MD

www.diahome.org

Disclaimer

The views expressed in this presentation are those of the speaker.

They do not necessarily reflect the “official” views of the Food and Drug Administration (FDA).

www.diahome.org

Overview

- Use of nonclinical microarray data in drug development
 - Efficacy (pharmacogenomics) vs. safety (toxicogenomics) data
- Industry Guidance on Pharmacogenomic Data Submissions (March 2005)
 - Requirements for microarray data submission to FDA (CFR vs. PG Guidance)
- Advantages to the use of genomics to test drugs in animal models of disease
- **Hypothetical** drug submission example
 - Use of microarray data to monitor drug-induced attenuation of ventricular remodeling associated with heart failure in animals
- Possible format for submission of microarray data from animal efficacy studies
- Conclusions

www.diahome.org

Use of Nonclinical Microarray Data in Drug Development

- Preclinical models of efficacy using *pharmacogenomics*:
 - What a drug is supposed to do
 - “Proof of concept” studies
 - Pharmacological mechanism of action
 - May be *under-utilized* in current drug development programs
 - Submission of tabulated efficacy data generally is not required (CFR)
- Preclinical safety assessment using *toxicogenomics*:
 - What a drug is not supposed to do
 - Active area in drug development
 - Used primarily to:
 - Screen candidate compounds for toxicity *early* in drug development
 - Provide insights into mechanisms of toxicity
 - Predict possible human toxicity
 - Reduce cost (\$) of drug failures
 - Submission of tabulated safety data generally is required (CFR)

www.diahome.org

Guidance for Industry: Pharmacogenomic Data Submissions (March 2005)

- Submission of pharmacogenomic data during the **IND** phase:
 - “Adequate information about pharmacologic and toxicological studies of the drug involving laboratory animals or *in vitro*, on the basis of which the sponsor has concluded that it is reasonably safe to conduct the proposed clinical investigations.” (**21 CFR 312.23**)
 - “A sponsor is using the test results to support scientific arguments pertaining to, for example, the pharmacologic mechanism of action...” (**PG Guidance**)

www.diahome.org

Guidance for Industry: Pharmacogenomic Data Submissions (March 2005)

- Submission of pharmacogenomic data during the **NDA** phase:
 - “The [NDA] application is required to contain reports of all investigations of the drug product sponsored by the applicant, and all other information about the drug product pertinent to an evaluation of the application that is received or otherwise obtained by the applicant from any source.” (**21 CFR 314.50**)
 - “Submit reports of pharmacogenomic test results that constitute known valid, or probable valid, [but not *unestablished valid*] biomarkers for physiologic, pathophysiologic, pharmacologic, toxicologic, or clinical states or outcomes in the relevant species.” (**PG Guidance**)

www.diahome.org

To Submit or Not to Submit: That is the Question

- “Companies don’t have to submit any data on nonclinical efficacy biomarkers” (J. Woodcock; *in Environ. Health Perspect.*, Aug 2004)
- However, compelling scientific arguments can be made to the use of genomic data to test drugs in animal models of human disease
- Therefore, decision by sponsor to submit nonclinical genomic efficacy data should be based on whether such data will strengthen their case for further study in humans

www.diahome.org

Advantages to the Use of Genomics to Test Drugs in Animal Models of Disease

- Characterize a specific disease process and its progression at the molecular level
- Monitor attenuation or regression of disease by specific therapeutic treatment at the molecular level
- Genomic profiling used in animals to monitor drug treatment of a specific disease process is only one component of the package of primary pharmacodynamic studies submitted in support of a drug application

www.diahome.org

Advantages to the Use of Genomics to Test Drugs in Animal Models of Disease (cont)

- Industry concerns regarding submission of nonclinical efficacy data and its use by regulators should not deter conduct of such studies if microarray can be used to support specific scientific arguments
 - **Case Example:** Treatment (or attenuation) of ventricular remodeling associated with heart failure in an animal model

www.diahome.org

Attenuation of Ventricular Remodeling Associated with Heart Failure

- *Brief* background on heart failure in humans
- Maladaptive responses of a failing heart
 - Myocardial hypertrophy
 - Ventricular remodeling
 - *Possible* role of matrix metalloproteinases
- Attenuation of ventricular remodeling with specific treatment
- Usefulness of microarray data to first characterize a specific disease process, then monitor therapeutic attenuation of that disease using appropriate animal models

www.diahome.org

Heart Failure (HF)

- HF develops when the heart fails to pump sufficient blood to supply the metabolic needs of the body's tissues
- Results in significant mortality and morbidity
 - In U.S., annual health care cost exceeds **\$30 billion** annually
- HF represents a common final-stage of a complex disease process with several possible etiologies:
 - Prolonged increases in blood pressure or volume
 - Mutations resulting in various familial forms of cardiomyopathies (e.g., dilated and hypertrophic)
 - Loss of myocytes due to exposure of toxic substances (e.g., doxorubicin)
 - Altered cardiac rhythm or conduction disturbances
 - Ischemia resulting from coronary artery disease or myocardial infarction (*most common*)

www.diahome.org

Animal Models of HF

Animal Models of Heart Failure

Techniques	Species
Naturally Occurring Models	
Dilated cardiomyopathy	Hamster,* dog, turkey
Salt-sensitive hypertension	Rat
Experimentally Induced Models	
Myocardial ischemia	
Coronary ligation	Rat,* dog, pig, rabbit
Coronary embolism	Dog, pig
Electrical shock	Dog
Chronic rapid cardiac pacing	
Ventricular pacing	Dog,* pig, rabbit
Supraventricular pacing	Dog,* rabbit
Pressure overload	
Aortic banding	Rat, guinea pig
Pulmonary artery banding	Mouse, rat, cat, dog, pig
Volume overload	
Arteriovenous shunt	Rat, dog
Mitral regurgitation	Dog
Aortic regurgitation	Rabbit
Toxic cardiomyopathy	
Doxorubicin	Rat, rabbit, dog, pig
Alcohol	Rat, turkey
Genetically altered animals	
Dilated cardiomyopathy	Mouse

*Most frequently used models.
 From Hongo M, Ryoke T, Ross J Jr: Animal models of heart failure: Recent developments and perspectives. Trends Cardiovasc Med 1997; 7(5):161-167.

(From: Hunter JJ *et al.*, "Molecular and Cellular Biology of Cardiac Hypertrophy and Failure", Ch. 9, in Molecular Basis of Cardiovascular Disease, Chien KR (Ed.), WB Saunders Co., 1999.)

www.diahome.org

Gene Expression Studies from Failing Human Hearts

- Transcription profiles indicate possible genomic markers or molecular pathways in human HF:
 - Failing vs. non-failing hearts
 - Ischemic vs. non-ischemic cardiomyopathy
 - Dilated vs. hypertrophic cardiomyopathy
- Gene expression changes in human HF shown to be partly reversible following mechanical support with temporary ventricular assist device (Hall JL *et al.*, 2004)

www.diahome.org

Differentially-Expressed Genes: Failing (F) and Nonfailing (NF) Human Hearts

GenBank accession no.	Name	Mean of NF, average difference units ±SD	Mean of F, average difference units ±SD	Fold change
Up-regulated genes				
M31776	BNP	751 ± 367	5,956 ± 1,908	↑ 7.9
M54951	ANF	1,477 ± 1,228	6,249 ± 1,434	↑ 4.2
M25296	ANF precursor	2,108 ± 751	6,986 ± 1,137	↑ 3.3
M55998	α1 collagen type I	716 ± 319	2,667 ± 1,199	↑ 3.7
Z74616	Pro-α2 collagen type I	98 ± 79	485 ± 312	↑ 4.9
D13666	Osteoblast specific factor 2	40 ± 39	474 ± 281	↑ 12
U21128	Lumican	462 ± 158	1,753 ± 561	↑ 3.8
X06700	Pro-α1 collagen type III	156 ± 37	510 ± 229	↑ 3.3
Z19585	Thrombospondin-4	293 ± 101	1,040 ± 425	↑ 3.5
M92934	Connective tissue growth factor	246 ± 110	794 ± 435	↑ 3.2
Z24724	Poly(A) site DNA	196 ± 81	540 ± 114	↑ 2.7
U10550	GEM GTPase	131 ± 29	359 ± 114	↑ 2.7
M59287	CDC-like kinase 1	126 ± 45	341 ± 84	↑ 2.7
HG2755	T-plastin	87 ± 54	234 ± 98	↑ 2.7
L02950	Mμ-crystallin	1,327 ± 372	3,389 ± 648	↑ 2.6
Down-regulated genes				
M22430	Phospholipase A2	1,563 ± 673	304 ± 92	↓ 5.1
L19267	Myosin protein kinase	1,072 ± 709	217 ± 186	↓ 4.9
K02765	Complement component 3	1,071 ± 332	230 ± 110	↓ 4.6
HG3945	Phospholipid transfer protein	409 ± 130	12 ± 203	↓ 35
M26311	Cystic fibrosis antigen	294 ± 114	22 ± 68	↓ 13
X07315	Nuclear transport factor	255 ± 127	32 ± 152	↓ 7.8
M12963	Alcohol dehydrogenase I	723 ± 188	150 ± 239	↓ 4.8
M33195	Tc-ε-receptor γ-chain	316 ± 64	67 ± 64	↓ 4.7
S80437	Fatty acid synthase	259 ± 162	55 ± 108	↓ 4.6
M14539	Factor XIII subunit	915 ± 493	208 ± 614	↓ 4.4
M80359	MAP/microtubule affinity regulating kinase 3	319 ± 72	96 ± 112	↓ 3.3
U42031	Progesterone receptor- associated immunophilin	456 ± 167	169 ± 109	↓ 2.7
D17408	Calponin	1931 ± 1337	726 ± 2825	↓ 2.7

Genes are ranked by fold change and expression level.

(From: Tan FL *et al.*, PNAS 2002; 99:11387-11392.)

www.diahome.org

Differentially-Expressed Genes (partial list): Ischemic (ICM) vs. Non-Ischemic (NICM) Cardiomyopathy in Humans

Table 2. Differentially expressed genes shared between ICM vs. NF heart and NICM vs. NF heart comparisons

Gene Symbol	Gene Name	ICM-NF		NICM-NF	
		Fold change	FDR	Fold change	FDR
Cell growth/maintenance					
HBA2	hemoglobin, alpha-2	4.3	0.50	2.7	0.1
HSAGL2	human alpha-globin gene	3.5	0.50	2.4	0.1
HBB	hemoglobin, beta	3.4	0.50	2.6	0.1
HBA2	hemoglobin, alpha-2	3.4	0.50	2.2	0.1
HBA1	hemoglobin, alpha-1	3.3	0.50	2.1	0.1
AF059180	mutant beta-globin gene	3.0	0.50	2.4	0.1
HBB	hemoglobin, beta	3.0	0.50	2.6	0.1
DUT	dUTP pyrophosphatase	2.2	0.50	2.2	0.1
RARRES1	retinoic acid receptor responder-1	-3.0	0.90	-2.2	0.5
Signal transduction					
PIK3R1	phosphoinositide 3-kinase, reg subunit, polypeptide-1	3.1	0.50	2.3	0.1
NPR3	atrial natriuretic peptide receptor C	3.1	0.50	2.5	0.1
CEBL	Cas-B β retroviral transforming sequence b	2.3	0.50	2.3	0.1
EDNRA	endothelin receptor type A	2.1	2.76	2.1	0.5
DKFZ56411922	adican	2.0	1.28	2.4	0.1
TNFRSF11B	tumor necrosis factor receptor superfamily, member-11b	-2.7	1.69	-2.0	1.1
SCYA2	small inducible cytokine A2	-3.5	0.90	-2.9	0.1
Metabolism					
EIF1AY	eukaryotic translation initiation factor-1A	2.2	0.50	2.2	0.6
KIAA0669	KIAA0669 gene product	2.2	0.50	3.2	0.1
SFPQ	splicing factor proline/glutamine rich	2.1	0.50	2.0	0.1
Nucleus					
PHLDA1	pleckstrin homology-like domain, family A, member-1	3.5	0.50	5.1	0.1
PHLDA1	pleckstrin homology-like domain, family A, member-1	3.3	0.50	4.9	0.1
ANP32E	acidic nuclear phosphoprotein 32 family, member E	2.0	0.50	2.7	0.1
Cell adhesion/cell communication					
COL21A1	collagen, type XXI, alpha-1	2.3	0.50	2.3	0.1
FCN3	ficollin-3	-3.2	0.90	-2.6	0.1

(From: Kittleson MM et al., *Physiol. Genomics* 2005; 21:299-307.)

www.diahome.org

Differentially-Expressed Genes (partial list): Dilated Cardiomyopathy (DCM) vs. Non-Failing Human Hearts

Table 3. Genes with altered expression as defined by score <0.025 or >0.975 and >1.5 fold change (50th percentile) in dilated cardiomyopathy

	Accession No.	Fold Change	Score
DCM Upregulated Genes			
Cell Division			
cell cycle gene RCC1	D00591	1.6(1.1,2.2)	0.0117
replication protein A 14-kDa subunit	L07493	1.5(1.2,1.9)	0.0041
Cell signaling/communication			
actin-related protein Arp3 (ARP3)	AF006083	1.7(1.1,2.3)	0.0123
ADP-ribosylation factor-like protein 1	L28997	1.7(1.1,2.3)	0.0135
atrial natriuretic peptide (ANP)	M30262	8.5(1.9,15.0)	0.0036
beta-arrestin 1	AF084940	1.6(1.2,1.9)	0.0014
folliculin-related protein (FRP)	D89537	1.6(1.2,2.0)	0.007
interferon alpha induced transcriptional activator	M97804	1.6(1.1,2.5)	0.0096
junctional adhesion molecule	U89915	1.5(1.1,1.9)	0.0073
lipocortin (annexin)	X05908	2.2(1.4,2.9)	0.0147
platelet/endothelial cell adhesion molecule-1	L34635	1.5(1.1,1.9)	0.007
receptor tyrosine kinase hek11	L36842	2.0(1.0,3.0)	0.0234
rhoHP1 (ras-like protein)	D85815	2.2(1.4,3.0)	0.0015
Cell structure/motility			
actin, beta cytoplasmic	M12481	1.6(1.1,2.1)	0.0153
collagen alpha-1 type 1	M55998	1.5(1.0,2.0)	0.0236
decorin	NM_001920	2.2(1.3,3.1)	0.0008
luniscan	NM_002345	2.1(1.3,3.2)	0.0141
myosin alkali light chain, atrial	M24121	2.1(1.2,3.0)	0.0035
tropomyosin TM30 (nm), cytoskeletal	X04588	1.6(1.1,2.1)	0.0097
Cell/organism defense			
calnexin	M94859	1.6(1.1,2.1)	0.0122
immunoglobulin heavy chain variable region V3-43	M99672	1.6(1.1,2.1)	0.0082
major histocompatibility locus class III regions	AF109905	2.0(1.2,2.9)	0.0084
myoglobin	M14603	1.8(1.2,2.4)	0.0044
T cell-specific protein	M21121	2.4(1.0,3.7)	0.0212
T-cell receptor beta chain	L36092	1.5(1.0,2.0)	0.0162

(From: Hwang J et al., *Physiol. Genomics* 2002; 10:31-44.)

www.diahome.org

Differentially-Expressed Genes (partial list): Hypertrophic Cardiomyopathy (HCM) vs. Non-Failing Human Hearts

Table 4. Genes with altered expression as defined by score <0.025 or >0.975 and >1.5 fold change (50th percentile) in hypertrophic cardiomyopathy

	Accession No.	Fold Change	Score
<i>HCM upregulated Genes</i>			
Cell signaling/communication			
atrial natriuretic peptide (ANP)	M30262	9.2(3.2,15.1)	0.0011
follicle-stimulating protein (FRP)	D89937	1.7(1.1,2.3)	0.0127
GDP-dissociation inhibitor rab	D13888	1.5(1.1,1.9)	0.0142
interleukin-1 receptor-associated kinase	L76191	1.7(1.0,2.4)	0.0231
lectin P35	D63158	1.5(1.0,1.9)	0.0245
platelet-activating factor receptor	P25105	1.5(1.2,1.8)	0.0038
RAB7, member RAS oncogene family-like 1	D84488	1.8(1.1,2.1)	0.0112
voltage-dependent anion channel	L06132	1.8(1.0,2.2)	0.0187
Cell structure/motility			
20-kDa myosin light chain	J02854	2.1(1.3,2.8)	0.0033
actin, beta cytoplasmic	M12481	1.5(1.1,1.9)	0.0129
decorin	NM_001920	1.7(1.2,2.2)	0.014
desmin	U59167	1.8(1.1,2.1)	0.0003
dysfemin beta heavy chain B2HC	AB012308	1.5(1.2,1.8)	0.0041
Cell/organism defense			
90-kDa heat-shock protein	X15183	1.7(1.3,2.1)	0.008
heat shock protein (hsp40) homolog	U40992	1.7(1.2,2.3)	0.0062
heat shock protein HSP70	X51757	1.9(1.1,2.8)	0.0163
Protein/gene expression			
DBP2 for ATP-dependent RNA helicase	AB001601	1.5(1.1,1.8)	0.009
elongation factor 2 (EF-2)	X51466	1.7(1.2,2.1)	0.0079
ribosomal protein L10	P27635	1.6(1.0,2.2)	0.0221
ribosomal protein L12	D28443	1.8(1.1,2.5)	0.0128
ribosomal protein L12	L05505	1.7(1.1,2.4)	0.0136
ribosomal protein L39 homolog	L05096	1.5(1.0,1.9)	0.0171
ribosomal protein S17	M13932	1.7(1.2,2.2)	0.0064

(From: Hwang J et al., *Physiol. Genomics* 2002; 10:31-44.)

www.diahome.org

Differentially-Expressed Genes (partial list): Before vs. After Mechanical Unloading in Failing Human Hearts

Table 2. SAM table listing statistically significant genes in 19 paired human heart samples following mechanical unloading

Gene Name	Gene Description	GenBank Accession	Median Paired Fold Change
<i>Downregulated in post</i>			
212298_at	neuropilin-1	BE620457	1.52
203666_at	stromal cell-derived factor 1	NM_000609.1	1.69
218351_at	hypothetical protein FLJ20502	NM_017845.1	1.45
211671_s_at	nuclear receptor subfamily 3, group C, member 1	U01351.1	1.32
204284_at	protein phosphatase 1, regulatory (inhibitor) subunit 3C	N26005	2.13
209821_at	DVS27-related protein	AB024518.1	1.64
214761_at	OLF-1/EBF associated zinc finger gene	AW149417	1.47
206404_at	fibroblast growth factor 9 (glia-activating factor)	NM_002010.1	1.59
219436_s_at	endomucin	NM_016242.1	1.23
206462_at	glutaredoxin (thioltransferase)	NM_002064.1	1.33
205501_at	Homo sapiens cDNA FLJ25677 fls	AI143879	1.32
206201_s_at	mesenchyme homeo box 2	NM_005924.1	1.65
212989_at	mob protein	AI377497	1.28
219806_s_at	FMS protein	NM_020179.1	1.29
208131_s_at	prostaglandin I2 (prostaglandin) synthase	NM_000961.1	1.54
207332_s_at	transferrin receptor (p90, CD71)	NM_003234.1	1.47
205571_at	lipoyltransferase	NM_015929.1	1.37
202595_s_at	leptin receptor overlapping transcript-like 1	AF161461.1	1.2
203337_s_at	integrin cytoplasmic domain-associated protein 1	NM_004763.1	1.32
202687_s_at	tumor necrosis factor (ligand) superfamily, member 10	U57059.1	1.57
205047_s_at	asparagine synthetase	NM_001673.1	1.52
205150_s_at	KIAA0644 gene product	AV724192	1.61
<i>Upregulated in post</i>			
203543_s_at	basic transcription element binding protein 1	NM_001206.1	1.92
212665_at	DKFZP434J214 protein	AL556438	1.95
205883_at	zinc finger protein 145 (Kruppel-like)	NM_006006.1	2.6
207513_s_at	zinc finger protein 189	NM_003452.1	2
204152_s_at	forkhead box O3A	NM_001455.1	2.07

(From: Hall JL et al., *Physiol. Genomics* 2004; 17:283-291.)

www.diahome.org

Adaptive Mechanisms in HF to Maintain Pumping Function

- Myocardial hypertrophy to augment mass of contractile tissue
 - Complex intracellular signaling pathways
 - Suitable for microarray and pathway analysis of transcriptional profiles
- Increased release of catecholamines to enhance contractility
- Activation of the renin-angiotensin-aldosterone system to maintain arterial pressure
- In short-term, adaptive mechanisms in HF can be effective in maintaining normal cardiac function
- In long-term, heart's ability to compensate is finite, and these adaptive processes often result in adverse consequences (i.e., ventricular remodeling)

www.diahome.org

Ventricular Remodeling

- Adverse adaptive response following myocardial injury (e.g., infarction)
- Response initially adaptive:
 - Cardiac hypertrophy, fibrosis, and degradation and deposition of extracellular matrix components
- Progressive remodeling becomes a *maladaptive* process leading to significant morbidity and mortality:
 - Collagen important for mechanical stability of heart degraded by increased matrix metalloproteinase (MMP) activity
 - Replaced by fibrous intercellular deposits of poorly-linked collagen
 - Leads to weakening and dilatation of left ventricular (LV) wall

www.diahome.org

Role of Matrix Metalloproteinases (MMP) in Heart Failure

- Studies have shown increased protein expression and activation of MMPs (e.g., collagenases and gelatinases that are transcriptionally regulated by various inflammatory cytokines, hormones, and growth factors) during myocardial remodeling processes:
 - ACE inhibition suppressed increase in MMPs and prevented LV dilatation and systolic dysfunction in Dahl salt-sensitive rats with hypertensive HF (Sakata Y *et al.*, 2004)
 - Significant induction of MMPs in local region of infarction induced by coronary artery ligation in sheep (Wilson EM *et al.*, 2003)
 - TNF- α contributes to myocardial remodeling through induction of MMPs, and TNF- α blocking protein attenuates remodeling in a chronic pacing model of HF in dogs (Bradham WS *et al.*, 2002)
 - Increased MMP protein in hearts and blood from humans with recent MI or with HF (Jones *et al.*, 2003; Polyakova V *et al.*, 2004)

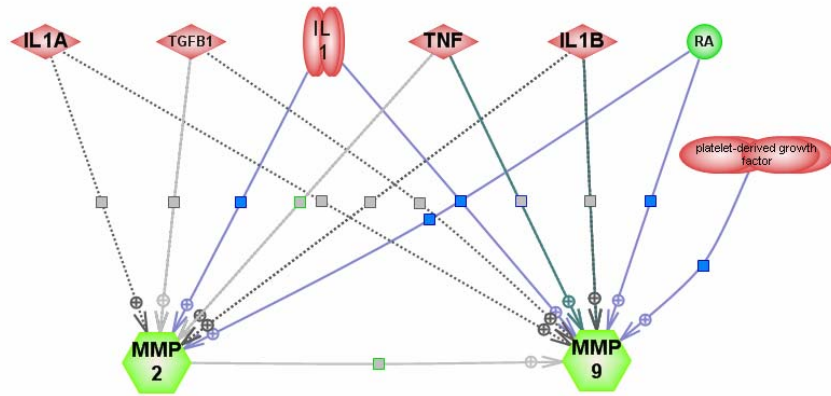
www.diahome.org

Role of Matrix Metalloproteinases (MMP) in Heart Failure

- Overall, these studies suggest:
 - A possible mechanistic explanation for role of MMPs in HF and their transcriptional regulation by inflammatory cytokines, hormones, and growth factors
 - Targeted pharmacological inhibition of MMPs (or their upstream transcriptional regulators) might have beneficial effects on attenuating the adverse ventricular remodeling process that follows MI
 - Microarray data including pathway analysis (e.g., hypertrophy, MMP expression, and fibrosis in HF) offers the possibility to monitor progression of HF, remodeling, and its attenuation with drug therapy in animals
 - ***Such data are difficult to obtain from human hearts***

www.diahome.org

Example of Pathway Analysis: Transcriptional Regulators of MMP



(Courtesy: Adriadne Genomics; Rockville, MD)

www.diahome.org

Possible Format for Submission of Microarray Data from Animal Efficacy Studies

- Tables of genes with *statistically significant* alterations in expression (i.e., differentially-expressed genes):
 - Gene variables include:
 - Gene name
 - GeneBank accession number
 - Gene function or role in cellular pathway, if known
 - Paired mean fold-change between:
 - Normal vs. diseased animals (i.e., for characterizing disease)
 - Treated vs. untreated diseased animals (i.e., for monitoring treatment)
- Figures of pathway analysis, if relevant

www.diahome.org

Conclusions

- Use of efficacy (i.e., *pharmacodynamic*) genomic data in animals can provide strong scientific support for further drug development in humans
 - “Proof of principle” studies
 - Pharmacological mechanism of action
 - Specific therapeutic treatment is acting on specific molecular pathway(s) associated with specific disease process
- Decision by sponsor to submit *nonclinical* genomic efficacy data should be based on whether such data will strengthen their case for further study in humans
- Refer to Pharmacogenomics Guidance (March 2005) for specific submission requirements (i.e., complete vs. abbreviated reports, or study synopses) and for information regarding VGDSs